Intracellular tyrosine kinases as novel targets for anti-fibrotic therapy in systemic sclerosis

Distler, J H W ; Distler, O

Abstract: Tissue fibrosis is a major cause of death in SSc, but therapies that target selectively fibrosis are not yet available for routine clinical use. Recent pre-clinical studies suggest that selective tyrosine kinase inhibitors that target c-Abl, PDGF receptor or Src kinases might be promising targets for anti-fibrotic approaches. Dual inhibition of c-Abl and PDGF receptor by imatinib and nilotinib, and inhibition of Src kinases either selectively by SU6656 or in combination with c-Abl and PDGF by dasatinib exerted potent anti-fibrotic effects. Imatinib, nilotinib, dasatinib and SU6656 reduced dose-dependently the synthesis of extracellular matrix protein in human dermal fibroblasts in vitro and prevented fibrosis in the mouse model of bleomycin-induced skin fibrosis. Clinical data from patients with chronic myelogenous leukaemia suggest that imatinib, nilotinib and dasatinib are well tolerated. Based on the promising pre-clinical data, imatinib is currently evaluated in clinical trials for the treatment of fibrosis in SSc and trials with other tyrosine kinase inhibitors are in preparation

DOI: https://doi.org/10.1093/rheumatology/ken276

Rheumatology 2008;47:v10–v11 doi:10.1093/rheumatology/ken276

Intracellular tyrosine kinases as novel targets for anti-fibrotic therapy in systemic sclerosis

J. H. W. Distler[1] and O. Distler[2]

Tissue fibrosis is a major cause of death in SSc, but therapies that target selectively fibrosis are not yet available for routine clinical use. Recent pre-clinical studies suggest that selective tyrosine kinase inhibitors that target c-Abl, PDGF receptor or Src kinases might be promising targets for anti-fibrotic approaches. Dual inhibition of c-Abl and PDGF receptor by imatinib and nilotinib, and inhibition of Src kinases either selectively by SU6656 or in combination with c-Abl and PDGF by dasatinib exerted potent anti-fibrotic effects. Imatinib, nilotinib, dasatinib and SU6656 reduced dose-dependently the synthesis of extracellular matrix protein in human dermal fibroblasts *in vitro* and prevented fibrosis in the mouse model of bleomycin-induced skin fibrosis. Clinical data from patients with chronic myelogenous leukaemia suggest that imatinib, nilotinib and dasatinib are well tolerated. Based on the promising pre-clinical data, imatinib is currently evaluated in clinical trials for the treatment of fibrosis in SSc and trials with other tyrosine kinase inhibitors are in preparation.

Key words: Imatinib, Dasatinib, Nilotinib, c-Abl, Src, Plate-derived growth factor, Transforming growth factor-β, Scleroderma, Fibrosis, Bleomycin.

Tissue fibrosis is a major cause of morbidity and mortality in SSc. Anti-fibrotic therapies that target selectively the activation of fibroblasts and the increased production of extracellular matrix (ECM) are not yet available. Recently, selective inhibitors of intracellular tyrosine kinases have been evaluated as novel anti-fibrotic approaches.

Imatinib mesylate (STI571, Gleevec®/Glivec®, Novartis) is a small molecule tyrosine kinase inhibitor that binds competitively to the ATP-binding pocket of abelson kinase (c-Abl) and thereby blocks efficiently its tyrosine kinase activity, which requires transition of ATP into ADP and phosphorylation of target proteins. c-Abl is an important downstream signalling molecule of TGF-β and PDGF [1]. In cells deficient for c-Abl, the induction of ECM proteins by TGF-β is strongly decreased. Of note, the activation of c-Abl by TGF-β is independent of Smad3 as the stimulation of c-Abl by TGF-β was not reduced in cells deficient for Smad3. In addition to its effects on c-Abl, imatinib mesylate interferes also with PDGF signalling by blocking the tyrosine kinase activity of PDGF receptors. Imatinib inhibits also the tyrosine kinase activity of the gene product of the proto-oncogene c-kit and of c-fms. Thus, imatinib targets simultaneously and selectively two major pro-fibrotic pathways activated in SSc.

Imatinib is widely used for the treatment of bcr-Abl-positive chronic myelogenous leukaemia and gastrointestinal stromal tumours with more 100 000 patients treated so far. Imatinib possesses favourable pharmacokinetic properties as it is well absorbed after oral administration and has to be taken only once daily.

We could demonstrate that imatinib strongly inhibited the synthesis of collagen 1a1, collagen 1a2 and fibronectin-1 by dermal fibroblasts by up to 90% in pharmacologically relevant concentrations [2]. Furthermore, treatment with imatinib prevented efficiently the development of fibrosis in the mouse model of bleomycin-induced dermal fibrosis with reduced dermal thickness, number of myofibroblasts and decreased synthesis of ECM in lesional skin. Smaller clinical case series suggested also that treatment of patients with chronic myeloid leukaemia (CML) might lead to a regression of concomitant bone marrow fibrosis [3]. While the mechanisms of bone marrow fibrosis are different from skin and organ fibrosis in SSc, the anti-fibrotic effect of imatinib did not correlate with the cytogenetic response, suggesting that the anti-fibrotic effects were independent from the suppression of cancer cells [3]. Imatinib mesylate might therefore not only prevent the progression of fibrosis in SSc patients, but might also be effective for the treatment of established fibrosis. This reflects the clinical situation, where patients usually present with at least partially established fibrosis.

Most recently, nilotinib (Tasigna®, Novartis) and dasatinib (Spyrcel®, Bristol-Myers Squibb), two novel inhibitors of Abl-kinases and PDGF receptors have been approved for the treatment of bcr-Abl-positive chronic myelogenous leukaemia with resistance or intolerance to imatinib. Nilotinib and dasatinib are also small molecule tyrosine kinase inhibitors, which can be administered orally [4, 5]. Like imatinib, nilotinib inhibits selectively the tyrosine kinase activity of Abl-kinases, PDGF receptor and c-kit. Dasatinib targets additionally the structurally related family of Src kinases. Both, nilotinib and dasatinib inhibit the activity of Abl significantly more potent than imatinib and are effective in most cell lines resistant to imatinib. The spectra of adverse effects of dasatinib and nilotinib differ from that of imatinib and patients with intolerance to imatinib can often be switched safely to nilotinib or dasatinib. We demonstrated recently that dasatinib and nilotinib both potently inhibit the production of ECM proteins in dermal fibroblasts and the development of fibrosis upon bleomycin challenge [6]. Thus, dasatinib and nilotinib might be interesting candidates for the treatment of patients who cannot tolerate imatinib.

This might be particular due for the combined Abl/Src inhibitor dasatinib, because we could demonstrate recently that Src kinases play an important role for the synthesis of ECM in SSc [7]. Src kinases are activated by pro-fibrotic cytokines such as TGF-β or PDGF in SSc fibroblasts. Inhibition of Src kinases by chemical inhibitors or overexpression of a dominant negative mutant of *Src* and the endogenous antagonist csk reduced strongly the synthesis of ECM proteins by dermal fibroblasts. Furthermore, treatment of mice with the specific inhibitor of Src kinases SU6656 reduced dose-dependently the dermal thickness and the collagen content in lesional skin in the mouse model of bleomycin-induced dermal fibrosis. Thus, Src kinases might also represent interesting novel targets for anti-fibrotic therapeutic approaches in SSc.

Abl kinase inhibitors have been shown to be well tolerated in clinical trials with CML patients with adverse side effects leading to the discontinuation of the drug in <1% of the patients. However, mild side-effects are frequent and outside of clinical trials, up to 30% of the patients discontinue imatinib because

[1]Department for Internal Medicine 3 and Institute for Clinical Immunology, University of Erlangen-Nuremberg, Erlangen, Germany and [2]Department of Rheumatology, Center of Experimental Rheumatology and Center of Integrative Human Physiology, University Hospital Zurich, Zurich, Switzerland.

Submitted 30 April 2008; accepted 19 June 2008.

Correspondence to: J. H. W. Distler, Department of Internal Medicine 3, University of Erlangen-Nuremberg, Germany. E-mail: joerg.distler@uk-erlangen.de

of adverse effects. The major adverse events responsible for discontinuation are dose-dependent and include oedema, muscle cramps, diarrhoea and bone marrow toxicity. Furthermore, Abl-kinase inhibitors might induce congestive heart failure. Recently, 10 patients have been reported, who developed congestive heart failure while on imatinib [8]. Histological evaluation of cardiac sections suggested a toxic cardiomyopathy. However, the interpretation of this report is complicated by the fact that the majority of patients reported with congestive heart failure while on imatinib had pre-existing cardiac disease or cardiovascular risk factors and that cardiotoxicity occurred only in 10 patients out of more than 100 000 patients treated with imatinib. Nevertheless, this potential side-effect needs special attention in the ongoing clinical trials in patients with SSc.

A particular concern of the use of tyrosine kinase inhibitors in SSc are anti-angiogenic side-effects. Vascular disease is a major feature of SSc and might be further aggravate by drugs that interfere with angiogenesis. Imatinib, nilotinib and dasatinib all inhibit the tyrosine kinase activity of PDGF receptor. As PDGF signalling is crucial for pericyte survival and as pericyte coverage is essential to stabilize new vessel, inhibition of PDGF receptor tyrosine kinase activity might inhibit angiogenesis [9]. However, PDGF signalling is up-regulated and overactivation of pericytes might play a role for both the vascular and fibrotic disease in SSc. Moreover, PDGF plays a crucial role in the pathogenesis of pulmonary arterial hypertension. Thus, treatment with the aforementioned tyrosine kinase inhibitors might exert beneficial effects by decreasing PDGF signalling to normal levels. Src kinases have also been implicated in endothelial cell functions and angiogenesis. VEGF activates *Src* in avian endothelial cells and overexpression of a dominant negative *Src* mutant inhibited VEGF-induced vascular permeabilization and angiogenesis. Thus, Src mediates VEGF-induced angiogenesis under normal circumstances. However, an uncontrolled overexpression of VEGF has been demonstrated in SSc patients. The uncontrolled overexpression of VEGF has deleterious rather than beneficial effects on angiogenesis that might further aggravate the vascular disease in SSc. Inhibition of Src signalling might therefore help to control the dysregulated activation of VEGF signalling and its negative effects on angiogenesis in SSc. Taken together, the net effect of Abl/PDGFR and Src inhibitors on angiogenesis in SSc is unclear and needs further investigation.

The mechanisms for tissue fibrosis and wound healing are similar in that fibroblast activation and increased production of ECM play central roles in both processes. Thus, anti-fibrotic therapies might also inhibit wound healing. Indeed, imatinib mesylate has recently been shown to interfere with the recruitment of pericytes and fibroblasts and wound closure in murine model of wound healing [10].

In summary, several lines of evidence suggest that Abl/PDGFR- and Src kinase inhibitors such as imatinib, nilotinib, dasatinib and SU6656 inhibit rather specifically fibroblast activation and synthesis of ECM and exert potent anti-fibrotic potentials. First clinical trials with imatinib mesylate for the treatment of fibrosis in SSc are currently ongoing and trials with other tyrosine kinase inhibitors are in preparation. The results of these trials might have great impact on the treatment of fibrotic diseases.

Rheumatology key messages

- Imatinib mesylate is a small molecule tyrosine kinase inhibitor that blocks TGF-β and PDGF signalling pathways.
- Imatinib mesylate showed potent anti-fibrotic affects in pre-clinical models including the bleomycin skin fibrosis model.
- Other clinically available c-Abl-kinase inhibitors with similar effects in pre-clinical fibrosis models include dasatinib and nilotinib
- c-Abl-kinase inhibitors are well tolerated in oncological diseases such as bcr-Abl-positive chronic myelogenous leukaemia with relatively rare severe side-effects, but rather frequent mild side-effects.

Acknowledgements

Dr Jörg Distler would like to acknowledge the grants they have received–ELAN grant 53410022 of the University Erlangen-Nuremberg and grant A20 of the Interdisciplinary Center of Clinical Research (IZKF) in Erlangen and Career Support Award of Medicine of the Ernst Jung Foundation.

Supplement: This paper forms part of the supplement entitled 'Update in systemic sclerosis'. This supplement was supported by an unrestricted grant from Encysive.

Disclosure statement: J.H.W.D. has received materials from Novartis and Bristol-Myers Squibb, is a member of a speakers' bureau for Actelion and Encysive and has received funding from Novartis. O.D. has received research grants from Encysive, served as a consultant for Encysive, Actelion and Array Biopharma and has received honoraria from Encysive, Actelion, Array Biopharma and Ergonex.

References

1 Daniels CE, Wilkes MC, Edens M *et al.* Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents bleomycin-mediated lung fibrosis. J Clin Invest 2004;114:1308–16.
2 Distler JH, Jungel A, Huber LC *et al.* Imatinib mesylate reduces production of extracellular matrix and prevents development of experimental dermal fibrosis. Arthritis Rheum 2007;56:311–22.
3 Bueso-Ramos CE, Cortes J, Talpaz M *et al.* Imatinib mesylate therapy reduces bone marrow fibrosis in patients with chronic myelogenous leukemia. Cancer 2004;101:332–6.
4 Kantarjian H, Giles F, Wunderle L *et al.* Nilotinib in imatinib-resistant CML and philadelphia chromosome-positive ALL. N Engl J Med 2006;354:2542–51.
5 Talpaz M, Shah NP, Kantarjian H *et al.* Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med 2006;354:2531–41.
6 Akhmetshina A, Dees C, Skhirtladze C *et al.* Dasatinib and nilotinib, two inhibitors of c-Abl and PDGF receptor signaling, for the treatment of dermal fibrosis. FASEB J 2008,Mar 7;[Epub ahead of print].
7 Skhirtladze C, Distler O, Dees C, et al. Src kinases in systemic sclerosis: central roles in fibroblast activation and in skin fibrosis, Arthritis Rheum 2008, in press.
8 Kerkela R, Grazette L, Yacobi R *et al.* Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. Nat Med 2006;12:908–16.
9 Distler JH, Hirth A, Kurowska-Stolarska M, Gay RE, Gay S, Distler O. Angiogenic and angiostatic factors in the molecular control of angiogenesis. Q J Nucl Med 2003;47:149–61.
10 Rajkumar VS, Shiwen X, Bostrom M *et al.* Platelet-derived growth factor-beta receptor activation is essential for fibroblast and pericyte recruitment during cutaneous wound healing. Am J Pathol 2006;169:2254–65.